Botanica SERBICA

(2022) 46 (2): 239-252

DOI: https://doi.org/10.2298/BOTSERB2202239E
journal homepage: botanicaserbica.bio.bg.ac.rs

Original Scientific Paper

Investigation of phenolic compounds, *in vitro* antioxidant and enzyme inhibition activities of methanol and aqueous extracts of different parts of *Glaucosciadium cordifolium*

Nuraniye Eruygur[1*], Fatma Ayaz[1], Yavuz Bağcı[2], Hamide Filiz Ayyildiz[3], Esra Maltaş Çağil[4], Prairna Malik[5] and Ahmad Ali[5]

1 Department of Pharmacognosy, Selcuk University-Faculty of Pharmacy, 42250 Konya, Turkey
2 Department of Pharmaceutical Botany, Selcuk University - Faculty of Pharmacy, 42250 Konya, Turkey
3 Department of Analytical Chemistry, Selcuk University - Faculty of Pharmacy, 42250 Konya, Turkey
4 Department of Biochemistry, Selcuk University - Faculty of Pharmacy, 42250 Konya, Turkey
5 Department of Life Sciences, University of Mumbai, Vidyanagari, Santacruz (East), 400098 Mumbai, India
* Correspondence: nuraniye.eruygur@selcuk.edu.tr

ABSTRACT:
The present study was designed to evaluate the biological potentials and phenolic composition of different parts of *Glaucosciadium cordifolium*, which is less investigated and known as a wild endemic species to Turkey. The antioxidant activity of the plant was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azinobis-(3-Ethylbenzthiazolin-6-Sulfonic Acid) (ABTS), iron chelating capacity, and a β-carotene / linoleic acid emulsion assay. The total phenol and flavonoid contents of the plant were determined using the Folin-Ciocalteu and aluminum chloride methods, respectively. The study of the enzyme inhibition activity of the plant was carried out for acetylcholinesterase, butyrylcholinesterase, α-glucosidase, α-amylase, and tyrosinase. The antiglycation activity of the aqueous extract of the plant was evaluated using established methods such as browning, a Nitroblue-tetrazolium (NBT) assay, the 2,4-dinitrophenyl hydrazine (DNPH) method, a Congo red assay, and fluorescent Bovine Serum Albumin (BSA). The HPLC profiling of the phenolics revealed that 18 standard phenolic compounds were found in different amounts in various extracts of the plant parts. According to our bioactivity results, the methanol extract obtained from the flower parts of the plant contained higher amounts of phenolic compounds and flavonoids, which also demonstrated the highest DPPH radical scavenging activity. In addition, the methanol extracts obtained from the leaves and roots were found to be the most active extracts against the acetylcholinesterase enzyme, as well as moderately active against the tyrosinase enzyme. The antiglycation capacity of the extract followed this order: *G. cordifolium* leaves > stems > roots > flower. As a result, our study indicated that *G. cordifolium* extracts have strong antioxidant potential, good enzyme inhibitory effects and antiglycation potential. Further studies on *G. cordifolium* with in vivo bioassays need to be carried out to seek the importance of the plant in pharmaceutical techniques.

Keywords:
Glaucosciadium cordifolium, HPLC analysis, antioxidant activity, enzyme inhibition, antiglycation activity

UDC: 582.794.1:547.56:577.15

Received: 27 January 2022
Revision accepted: 05 July 2022

INTRODUCTION

Glaucosciadium cordifolium (Boiss) Burtt et Davis [syn. *Siler cordifoium* Boiss, *Laser cordifolium* (Boiss.) and *Peucedanum mucronatum* Thieb.] belongs to the Umbelliferae family, and is a monotypic plant which grows in the Mediterranean region and is close to the genus *Peucedanum* L. (Hedge & Lamond 1972). The ethnopharmacological review shows that the roots and leaves of the plant are used for stomach ailments, and as an aphrodisiac, and appetizer (Bulut *et al.* 2014). The chemical composition of the essential oil obtained from *G. cordifolium* showed that the major components were detected as limonene, α-pinene, and β-pinene (Başer *et al.* 2000; Karadoğan *et al.* 2015).

Medicinal aromatic plants have been used in the treatment of various diseases for many years. Investigating the close relationship between plants and human health has led to the discovery of many new herbal therapeutic agents, such as herbal medicines, multi-component herbal medicines and food supplements as an alternative to conventional medicines currently used to treat cardiovascular diseases, cancer, diabetes, and neurological disorders. There are more than 30,000 studies in the literature on medicinal plants used for the treatment of diabetes mellitus (DM), and as natural food supplements. These studies include *in vitro*, in vivo, and clinical investigations (Yikna & Yehualashet 2021). DM is one of the most serious and chronic diseases, and can be attributed to hyperglycemia, which is characterised by excessive glucose levels in the bloodstream. Alpha-glucosidase inhibitors (AGI) delay the absorption of carbohydrates by inhibiting α-glucosidase enzymes in the small intestine (Qurrat-ul-ain *et al.* 2017). Acarbose, miglitol, and voglibose are the drugs belonging to this group of α-glucosidase inhibitors, and only acarbose is available in Turkey. However, these drugs involve limited dosage forms and short usage due to side effects such as abdominal pain, swelling and diarrhoea (Salehi *et al.* 2013). Therefore, it is necessary to develop new AGI drugs which have fewer side effects and can be more easily tolerated by the patient.

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterised by decreased cognitive functions and the ability to maintain daily activities, behavioural changes, and psychiatric symptoms (Russo *et al.* 2015). Due to the general population living longer, the lack of effective treatment methods and the inability to diagnose the progress of disease, AD frequency has been increasing in recent years. AD is caused by the degeneration of cholinergic neuronal transmitters, especially the basal forebrain acetylcholinergic neurons. Acetylcholine is the neurotransmitter released at the end of the neuron. Acetylcholine is synthesized in specific cells called cholinergic neurons, using the synthetic enzyme, choline acetyltransferase, and acetyl coenzyme A and cholinic acid as substrates (Eruygur & Ayaz 2021). The acetylcholinesterase enzyme has been an attractive target for rational drug design and the discovery of inhibitors based on this mechanism. Although acetylcholinesterase inhibitors are widely used in the treatment of AD, they can cause severe side effects such as anorexia, diarrhoea, fatigue, nausea, sleep disorders, muscle cramps, and gastrointestinal, cardiovascular, respiratory, and genital system diseases (Rhee *et al.* 2001). These side effects have led researchers to seek new cholinesterase inhibitors with higher efficacy, fewer side effects, and higher bioavailability, especially obtained from natural resources.

Tyrosinase is a polyphenol oxidase with a binuclear copper active site and plays an important role in the enzymatic browning of fruits and vegetables in the formation of melanin pigments in mammals (Kubo *et al.* 2003). The hyperactivity of this enzyme leads to skin hyperpigmentation. For this reason, chemical substances with tyrosinase inhibitory activity have been used for the suppression of melanogenesis and can be used clinically for certain dermatological disorders associated with melanin hyperpigmentation (Abirami *et al.* 2014). Kojic acid is one of the most popular chemicals used as a bleaching agent in cosmetics, although it has been shown to have adverse effects in chronic usage, such as cytotoxic and mutagenic effects (di Petrillo *et al.* 2016). To avoid these side effects, it is more important to discover natural anti-tyrosinase agents instead of chemical substances.

Glycation is a nonenzymatic reaction in which the binding of the free amino (–NH2) group of biological macromolecules such as proteins and nucleic acids with a reducing sugar from carbohydrates or lipids forms the Schiff base thus providing a more stable form of Amadori product which undergoes dehydration, rearrangement, and cyclisation to finally generate advanced glycation end products AGEs (Aldini *et al.* 2013). This phenomenon consists of two stages - the early stage and the late stage. In the early and reversible stage, a Schiff base is formed due to the interaction of glucose with the –NH2 groups of protein, which rearranges to form a stable ketoamine adduct. This process is also termed as the Maillard reaction. Within a few days, Amadori products are formed by the rearrangement of the Schiff bases. These products are considered as early glycation products (Ahmad *et al.* 2014). The accumulated Amadori products further lead to the late stage of glycation, thereby causing an increase in AGEs. The number of AGEs has been described as including 3-deoxyglucosone (3DG), pyrraline, pentosidine, and N-ε- (carboxymethyl) lysine (CML). It has been observed that glycation is connected to a variety of diseases such as DM, furthermore to aging and age-related disorders, myocardial infarction, hypertrophy, and neurodegenerative diseases etc. (Singh *et al.* 2014). A wide array of proteins and enzymes are supposedly affected by glycation, leading to alterations in their structure and functions. Glycation results in the chemical modifications, conformation-

al changes and retardation of protein function (Roy *et al.* 2004). This, in turn, affects normal metabolic processes which causes a further decline in the effectiveness of the immune system and makes the individual more prone to diseases. The glycation process is toxic because of its ability to inhibit and alter specific characteristics of proteins as well as to generate reactive oxygen radicals (Ali *et al.* 2022). However, the mechanism of the glycation process is still not clear and it is not fully known which products are involved in these complications.

In the literature survey, we noticed that no biological activity of *G. cordifolium* has been reported until now. Therefore, in the present study we aimed to investigate the extracts of different parts of *G. cordifolium* for their *in vitro* antioxidant activity (DPPH, ABTS, the β-carotene/linoleic acid emulsion method, and iron chelating activity), enzyme (acetylcholinesterase, butyrylcholinesterase, α-glucosidase, α-amylase, and tyrosinase) inhibitory effects, and antiglycation activities, as well as to carry out qualitative and quantitative analyses of the phenolic compounds by means of spectroscopic determination of the total phenol and flavonoid contents and the HPLC method. The study results showed that *G. cordifolium*, which is of economic importance due to its chemical components, especially volatile compounds, may possess different biological activities. Our work will provide important scientific data for further bioassay-guided research into the active secondary metabolites from *G. cordifolium*.

MATERIALS AND METHODS

Plant material. *G. cordifolium* was collected from Ermenek, a district of the Karaman province, Turkey (C4 Karaman; roadside and hillside of Ermenek, 1200 m, 03.08.2018), and identified by Dr. Yavuz Bagcı, Faculty of Pharmacy, Selcuk University. A voucher specimen (Bagcı 4170) has been deposited in the herbarium of KNYA. The plant material was dried in the shade and powdered to prepare different extracts.

Preparation of the plant extracts. 10 g of powdered plant material (roots, leaves, stems, and flowers) was suspended in aqueous methanol (80%) at room temperature for 24 h. Then the extracts were filtered through Whatman filter paper. The addition of methanolic (80%) solvent was repeated two more times, then the solvents were evaporated under reduced pressure at 40°C to yield a thick paste of aqueous methanol extract. After obtaining the aqueous methanol extract, the remaining plant parts were extracted with distilled water by the same extraction procedure, and the filtrates were dried in a water bath to yield water extracts. All of the extracts were stored in air-tight glass bottles at 4°C before use and later re-dissolved in their respective solvents to give the desired concentrations for the various experiments.

Analysis of the phenolic content

Identification of the phenolic compounds by RP-HPLC. The phenolic compounds were evaluated by reversed-phase high-performance liquid chromatography (RP-HPLC, Agilent Technologies, Wilmington, DE, USA) using a G1311A model quaternary pump, a G1315B model diode array detector, a G1329A model automatic injector system, and a G1316A model thermostatic column compartment. The chromatographic data were recorded and processed by a ChemStation B.03.02-2008 data processor (Agilent Technologies, Wilmington, DE, USA). The DAD detector was set at a wavelength of 280 nm generally used for the simultaneous determination of different phenolic compounds.

For analysis, 20 mg of dry crude extract was dissolved in 1 mL of methanol and the injection volume of the sample solution was 10 µL. The separations were conducted at 30°C on an ACE 5 C18 (250 × 4.6 mm; 5 µm) column. The analysis was performed by gradient elution with a flow rate of 0.8 mL/min. The mobile phase was 0.1% acetic acid in water (A), 0.1% acetic acid in methanol (B) and 0.1% acetic acid in acetonitrile (C) with a gradient elution programme of 80:12:8 (A:B:C) at 0–8 min, 75:15:10 at 8–10 min, 70:18:12 at 10–24 min, 65:20:15 at 24–32 min, 50:35:15 at 32–40 min, 25:60:15 at 40–45 min, and then back to the mobile phase (80:12:8) to the regenerated column for 5 min. The samples and mobile phases were filtered through a 0.22 µm filter (Millipore Corp., Billerica, MA) prior to HPLC injection. Each fraction was analysed in triplicate. Gallic acid, 3,4-dihydroxy benzoic acid, catechin, chlorogenic acid, 4-hydroxy benzoic acid, 1,2-dihydroxy benzene, epicatechin, vanillic acid, caffeic acid, vanillin, p-coumaric acid, sinapic acid, trans-ferulic acid, ellagic acid, rutin, salicylic acid, quercetin, and kaempferol were used as the standards. Triplicate 10 µL injections were made for each standard solution to identify the reproducibility of the detector response at each concentration level. The phenolic compounds were identified and quantified by comparing their retention time and DAD spectral data to previously known injected standards. The amount of each phenolic compound was expressed as µg per mg of extract.

Total phenolic content determination. The total phenolic content of the extracts was determined using the Folin-Ciocalteu method described by Clarke *et al.* (2013). 10 µL from each extract (2 mg/mL) was mixed with 100 µL Folin-Ciocalteu reagent diluted ten times with water (1/10, v/v), and after 5 min of reaction, 100 µL sodium carbonate (7.5%) was added to each tube and the mixture was further incubated for 60 min. The absorbance of the samples was measured at 650 nm. The experiments were performed in triplicate. The standard curve was prepared using 1–1000 µg/mL solutions of gallic acid in DMSO. The total phenol content was expressed as mg gallic acid equivalents per g dry weight of the extract (mg GAE/g

DW). The gallic acid equation of the calibration curve is as follows: $y = 0.003x + 0.0578$; $r^2 = 0.998$.

Total flavonoid content determination. The aluminum chloride colorimetric method was used to determine the total flavonoid content in the extracts according to the method proposed by Yang *et al.* (2011). For calibration, serial diluted solutions of 0.0625, 0.125, 0.25, and 0.5 mg/mL were prepared from quercetin stock solution (1 mg/mL). The test solution (150 µL, 0.3 mg/mL) was prepared with ethanol which is mixed on an aliquot of 96 well plates with an equal volume of 2% $AlCl_3$. After incubation for 15 min at room temperature, the absorbance was read at 435 nm. The quantities of total flavonoids were calculated as mg quercetin equivalents per g dry weight of extract. The quercetin equation of the calibration curve is as follows: $y = 0.0068x + 0.0928$; $r^2 = 0.9982$.

In-vitro antioxidant activity

DPPH radical scavenging activity. The DPPH radical scavenging potential of the extracts was determined by the method proposed by Clarke *et al.* (2013). The 20 µL test solutions diluted with DMSO were mixed in a 96-well plate with 180 µL of DPPH solution at a concentration of 40 µg/mL prepared in methanol. After leaving the plates in the dark for 15 min, the absorbance was read at 517 nm with an Elisa reader. DMSO was used as the blank instead of the test sample, and a standard solution of quercetin in DMSO was run in parallel. The results were expressed as % DPPH scavenging effect using the following equation:

$$\%\ \text{DPPH Scavenging Effect} = (\text{Control Absorbance} - \text{Sample Absorbance})/\text{Control Absorbance} \times 100$$

The control absorbances are all solutions which do not contain the test substances, and the sample absorbance is the extract/quercetin absorbance. The IC_{50} was calculated from the scavenging activities (%) versus concentrations of the respective sample curve.

ABTS radical scavenging activity. The method suggested by Re *et al.* (1999) was used to determine the ABTS scavenging activities of the plant extracts. Test samples were prepared as in the DPPH method. ABTS radical stock solution was prepared by allowing 15 mL of 7 mM ABTS and 264 µL of 140 mM potassium persulfate solution to stand in the dark at room temperature for 16 h. The ABTS working solution was adjusted to obtain an absorbance of 0.70±0.02 at 734 nm by diluting with methanol just before the experiment. On a 96-well plate, 50 µL of sample solution was mixed with 100 µL of ABTS working solution. After the mixture was allowed to stand at room temperature for 10 min, the absorbance was then read at 734 nm. The ABTS + scavenging activities of the plant extracts were compared with BHT, and the percent inhibition was calculated using the formula below:

$$\text{ABTS+ radical scavenging activity (\%)} = (Abs_{control} - Abs_{sample})/Abs_{control} \times 100$$

The IC_{50} was calculated from the scavenging activities (%) versus concentrations of the respective sample curve.

Iron chelating activity. The iron chelating activities of the extracts were determined by their interaction with the formation of the ferrozine-Fe^{2+} complex (Chai *et al.* 2014). Briefly, 200 µL of 0.1 mM $FeSO_4$, 200 µL of extract and 400 µL of 0.2 mM ferrozine were mixed in a test tube and reacted at 25°C. The absorbance of the mixture was read after incubation for 10 min at 562 nm. EDTA was used as a positive control. The metal chelating capacity was calculated according to the following formula:

$$I\% = (A_{control} - A_{test})/A_{control} \times 100$$

where, $A_{control}$ is the absorbance of the tube without the test sample, and A_{test} is the absorbance of the test sample.

The IC_{50} was calculated from the iron chelating activities (%) versus concentrations of the respective sample curve.

β-Carotene / linoleic acid emulsion method. β-Carotene / linoleic acid emulsion capacity was monitored according to the method of Martins *et al.* (2015), and Pires *et al.* (2016), with minor modifications. The plant extracts and synthetic antioxidant substance, Trolox, were prepared in methanol at a concentration of 2 mg/mL. Briefly, 0.4 mg of β-carotene was dissolved in the flask by adding 2 mL of chloroform. After shaken vigorously, the chloroform was removed by a rotary evaporator at 40°C, then 40 mg of linoleic acid, 400 mg of Tween 80, and 100 mL of deionized water were added to the flask and vigorously shaken. The same procedure was repeated for the control solution which did not contain β-carotene. Trolox was used as a positive control. 200 µL plant extracts, BHA, BHT solutions and 4.8 mL of the prepared β-carotene emulsion solution were added to the test tubes. The mixture was incubated in a water bath at 50°C. The absorbance of the test tubes and the control solution were read at 470 nm immediately (t_0), and then at 120 minutes (t_{120}). The absorbance change rate, and therefore the % oxidation inhibition coefficients were calculated according to the following formula:

$$\%\ I = a / b \times 100$$

where a is the absorbance after 120 min of incubation and b is the initial absorbance of the mixture.

The 50% inhibition concentration of antioxidant activity was calculated from the % inhibition-extract concentration graph.

***In vitro* enzyme inhibition activity**

Acetylcholinesterase inhibition assay. The acetylcholinesterase inhibition activity was determined with a 96-well plate assay based on the method in our previous study (Eruygur *et al*. 2022). Briefly, 140 µL phosphate buffer (0.1 mM, pH 6.8), 20 µL of acetylcholinesterase (0.22 U/mL), and 20 µL of the extract dissolved in MeOH in various concentrations were added to each well and incubated for 30 min with mixing. After adding 10 µL of 0.5 mM 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB or Ellman's reagent), the reaction was started by the addition of 10 µL of 0.71 mM acetylthiocholine. The absorbance was measured at 412 nm with a microplate reader (Multiscan Go, USA). Galanthamine was used as a positive control, and methanol was used as a negative control.

Butyrylcholinesterase inhibition assay. The butyrylcholinesterase inhibitory activity was determined with a 96-well plate assay based on the method in our previous work (Eruygur *et al*. 2022). The assay was the same as the acetylcholinesterase inhibition assay. The only difference was the use of butyrylthiocholine as the substrate and horse serum butyrylcholinesterase (0.1 U/mL) as the enzyme. In the same manner, 10 µL butyrylthiocholine chloride (0.2 mM) was added to the mixture as the substrate. Galanthamine was used as the standard. The percentage of inhibition was calculated by comparing the reaction rates of the samples to the negative control in which methanol was used instead of the sample.

Alpha-glucosidase inhibition assay. The 96-well plate spectrophotometric method proposed by Telagari & Hullatti (2015) was modified to determine the α-glucosidase inhibitory activities of the extracts obtained from different parts of *G. cordifolium*. In this method, *p*-nitrophenyl α-D-glucopyranoside (PNPG) was used as the substrate. Briefly, 50 µL of 100 mM potassium phosphate buffer (pH 6.8), and 10 µL of α-glucosidase (EC 3.2.1.20, from *Saccharomyces cerevisiae*) solutions were prepared in the same phosphate buffer (1 U/mL) and 20 µL of extracts were added to a 96-well plate. The plate was kept at 37°C for 15 min. Next, 20 µL of 5 mM PNPG was added to each well. The plate was then incubated at 37°C for 20 min. The reaction was then terminated by the addition of $NaCO_3$ (50 µL, 0.1 mM). The absorbance was measured at 405 nm. Acarbose was used as a positive control. The α-glucosidase inhibition rate was calculated according to the following formula:

$$(\%) = [1- (A_{sample}-A_{s\text{-}blank}) / (A_{control}-A_{blank})]$$

Here, A_{sample} refers to the absorbance of the mixture containing the test sample, enzyme and substrate, $A_{s\text{-}blank}$ refers to the absorbance of the mixture containing the test sample and substrate, $A_{control}$ refers to the absorbance of the mixture containing the substrate and enzyme, and A_{blank} refers to the absorbance of the mixture containing only the substrate.

Alpha-amylase inhibition assay. The extracts were evaluated for their α-amylase inhibitory activity using the Caraway-Somogi iodine /potassium iodide method with minor modifications (Özek 2018). The porcine pancreatic amylase was prepared with a phosphate buffer at a concentration of 0.8 U/mL prior to the experiment. 0.05% starch solution was freshly prepared to use as the substrate. Acarbose was used as a positive control. Briefly, 50 µL of 20 mM phosphate buffer (pH 6.9), 50 µL of 0.8 U/mL of pancreas amylase enzyme solution and 25 µL of the extracts in different concentrations were added to a 96-well plate and incubated for 10 min at 37°C. Then, 50 µL of starch solution was added to the mixture and further incubated for 10 min. The reaction was then terminated by the addition of HCI (25 µL, 1 M). At the end, 100 µL of I_2/KI reagent was added to the mixture. The absorbance of the mixture was read for the sample and blank at 630 nm. The percentage inhibition of the α-amylase was calculated according to the formula given in the α-glucosidase inhibition assay.

Tyrosinase inhibition assay. The tyrosinase enzyme inhibitory activity of the *G. cordifolium* extracts was carried out as previously described (Jeong *et al*. 2009). Briefly, 100 µL of 100 mM phosphate buffer (pH 6.8), 20 µL of 250 U/mL of tyrosinase enzyme solution and 20 µL of the extract in different concentrations were added to a 96-well plate and incubated for 10 min. After adding 20 µL of 3 mM L-tyrosine as the substrate, the mixture was further incubated for 30 min at room temperature. The absorbance was measured at 492 nm by a microplate reader. Kojic acid was used as a positive control. The % inhibition value was calculated by the following formula:

$$\% \text{ Inhibition} = [1- (Aa-Ab) / (Ac-Ad)] \times 100$$

where, Aa is the absorbance of the test samples with the enzyme, Ab is the absorbance of the test sample without the enzyme, Ac is the absorbance of the enzyme solution without the test samples, and Ad is the absorbance without both the enzyme and test samples.

***In vitro* glycation of albumin**. For the evaluation of the antiglycation properties of the *G. cordifolium* extracts, an aqueous solution of BSA (10 mg/mL) was incubated along with the plant extracts (1 mg/mL) and fructose (100 mg/mL) in 0.1M potassium phosphate buffer (pH 7.4) containing 3 mmol/L sodium azide for the prevention of bacterial contamination at 37°C for 28 days in sealed tubes under sterile conditions (0.22 µm filter), hereafter referred to as the glycated sample. After incubation, the unbound protein was removed by dialysis against PBS, and the dialysate was used for further analysis.

Effects of the *G. cordifolium* extracts on browning. Browning was used as a simple colorimetric method for the assessment of the glycation process. The absorbance of the glycated sample in both the presence and the absence of the plant extracts was measured using a Shimadzu UV 1800 spectrophotometer at 420 nm and the relative percentage was calculated using BSA+ Fructose as the standard (100%) (Ali *et al.* 2017). The results are expressed as mean±SEM (n=3). Statistical significance was $p < 0.05$ when compared to BSA + Fructose.

Effects of the *G. cordifolium* extracts on fructosamine production. The production of fructosamine content was measured by the NBT assay as described by Banan & Ali (2016) with slight modifications. Firstly, 10 µL of the glycated samples was incubated with 100 µL of 0.5 mM NBT in 0.1 M carbonate buffer (pH 10.4) at 37°C for 15 min. Following incubation, 1 mL of the reaction mixture was made using 890 µL of distilled water and the absorbance was measured using a Shimadzu UV 1800 spectrophotometer at 530 nm.

Determination of protein carbonyl groups. The protein carbonyl groups in the glycated samples were assayed according to the DNPH method suggested by Meeprom *et al.* (2013) with slight modifications. Initially, 100 µL of the glycated samples was incubated with 400 µL DNPH (10 mM in HCL 2.5 M) in dark conditions for one hour at room temperature, followed by protein precipitation using 0.5 ml of 20% TCA (w/v). Then, the tubes were centrifuged at 10,000 rpm for 10 min at 4°C and the precipitate was washed three times in 500 µL of ethanol-ethyl acetate mixture (1:1 v/v) and dissolved in 100 µL of 6 M guanidine hydrochloride. The reaction mixture was then made up to 1 mL to obtain a sufficient volume for spectroscopic measurement at 370 nm using a Shimadzu UV 1800 spectrophotometer.

Determination of amyloid β-aggregation using Congo red assay. Aggregation in the glycated sample was measured using amyloid-specific Congo red dye according to Adisakwattana *et al.* (2014) with slight modifications. Congo red (100 µM) was prepared in PBS (pH 7.4) containing ethanol (10%, v/v). Initially, 50 µL of glycated BSA was incubated with 50 µL of 100 µM Congo red for 20 minutes at 25°C. The volume was increased to 1 mL with D/W and then the absorbance was measured using a Shimadzu UV 1800 spectrophotometer at 530 nm.

Estimation of total fluorescent AGEs in the presence of the *G. cordifolium* extract. The *in vitro* antiglycation activity of the plant extracts was examined by the BSA-fluorescent assay using a Cary Eclipse Fluorescence spectrophotometer (Varian). The fluorescent intensity was measured at excitation and emission wavelengths of 370 nm and 440 nm, respectively as described by Kumar *et al.* (2021).

Statistical analysis. The results of each experiment were represented as Mean ± SD. The analysis of variance (ANOVA) test was used to analyse the data for significance and means with significant differences were determined by using Duncan's test with SPSS (22.0) software, where the results with a p value ≤ 0.05 were accounted as statistically significant.

RESULTS

Identification of the phenolic compounds by RP-HPLC. Qualitative and quantitative analysis of the aqueous and methanol extracts of *G. cordifolium* were determined by RP-HPLC analysis. The regression analysis of the peak area ratio (*y*) vs. concentration (*x*) for the most abundant phenolic compounds is shown in Supplementary Table 1. The amounts of phenolic compounds detected in the samples are presented in Table 1. The chromatograms of the determined compounds are shown in Figs. 1 & 2. Linear regression analysis, using the least squares method, was used to evaluate the calibration curve of each analyte as a function of its concentration. The limit of detection (LOD) was estimated as $3.3S_b$/slope of the calibration curve, where S_b was the standard error of the intercept (*b*) (95% confidence limit). Concentrations of phenolics (mg/L) were above the corresponding limit of detection.

The DAD-spectra of the eluted compounds revealed that generally, the most abundant phenolics in all the extracts obtained from the plant parts were chlorogenic, caffeic, and salicylic acids. Among all the extracts analysed for their phenolic profiles, the highest amount of phenolic content was observed in the methanolic extract obtained from the flower parts of the plant, such as chlorogenic acid, ellagic acid, and salicylic acid with quantities of 21.78, 5.52, and 9.67 mg/g, respectively. The same compounds were determined in the leaves methanol extract as 7.87, 1.16, and 4.77 mg/g, while the highest phenolic compound content obtained from the extracts of the stems and roots was caffeic acid at 1.53–3.66 mg/g. On the other hand, among the three flavonoid compounds analysed, the rutin content was found to be higher in the methanol extract obtained from the flowers (3.52 mg/g extract), followed by the methanol extracts of the stems and leaves at 0.73 mg/g and 0.49 mg/g, respectively. Kaempferol was detected only in the methanol extract of the flower parts and the water extract of the leaves.

***In vitro* antioxidant activity**. The antioxidant properties of *G. cordifolium* were evaluated by various antioxidant assays and the results obtained in this study are shown in Table 2. The free radical scavenging activity of the extracts of different parts of *G. cordifolium* was measured by the DPPH and ABTS assays. Quercetin and BHT were used as the standards, respectively. The flower methanol extract exhibited the highest DPPH radical scavenging activity (IC_{50}: 0.25±0.005 mg/mL), followed by the stem

Table 1. The phenolic contents of the methanol and aqueous extracts of different parts of *G. cordifolium* (µg/mg, n=3)

Analyte	Retention time (min)	Samples							
		GCFM	GCFW	GCLM	GCLW	GCSM	GCSW	GCRM	GCRW
Gallic acid	4.69	0.345	-	0.325	0.283	1.045	-	0.572	0.330
3,4-dihydroxy benzoic acid	6.98	0.315	0.187	0.136	0.232	0.269	0.365	0.478	0.289
Catechin	7.97	0.148	0.106	0.088	0.130	0.061	0.124	0.098	0.069
Chlorogenic acid	8.79	21.78	0.165	7.866	0.073	0.182	0.071	0.187	0.692
4-hydroxy benzoic acid	10.65	0.095	-	-	-	-	-	-	0.312
1,2-dihydroxy benzene	11.09	0.173	0.221	-	0.148	0.119	0.217	0.195	0.830
Epicatechin	11.40	0.078	0.420	-	-	0.098	-	-	-
Vanillic acid	11.80	0.119	0.241	0.292	0.183	0.249	0.289	-	-
Caffeic acid	12.18	0.822	0.168	0.964	-	1.727	2.181	1.533	3.661
Vanillin	17.63	0.160	-	0.124	-	0.119	-	0.054	-
p-Coumaric acid	18.27	0.100	0.255	0.192	0.123	0.059	0.052	0.173	0.484
Sinapic acid	19.17	0.499	-	0.265	-	0.165	0.099	0.087	0.085
Trans-Ferulic acid	20.07	0.149	0.386	0.113	0.165	0.137	0.282	0.476	0.104
Ellagic acid	21.17	5.518	0	1.155	-	0	0	0	0.232
Rutin	22.40	3.520	0.085	0.486	-	0.731	-	0.082	-
Salicylic acid	32.88	9.667	-	4.767	-	1.640	0.072	0.702	0.056
Quercetin	36.26	0.064	0.188	0.245	-	0.282	0.399	0.290	-
Kaempferol	39.97	0.327	-	-	0.185	-	-	-	-

GCFM: methanol extract of flowers; GCFW: aqueous extract of flowers; GCLM: methanol extract of leaves; GCLW: aqueous extract of leaves; GCSM: methanol extract of stems; GSFW: aqueous extract of stems; GCRM: methanol extract of roots; GCRW: aqueous extract of roots

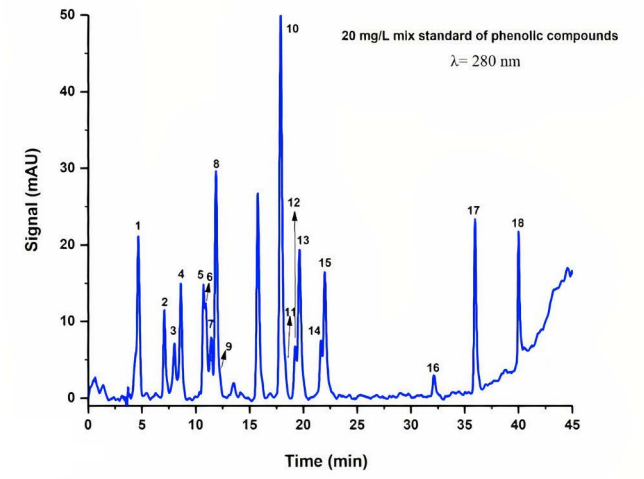

Fig. 1. HPLC Chromatogram of mixed standard phenolic compounds (peaks 1: gallic acid; 2: 3,4-dihydroxy benzoic acid; 3: Catechin; 4: Chlorogenic acid; 5: 4-hydroxy benzoic acid; 6: 1,2-dihydroxy benzene; 7: Epicatechin; 8: Vanillic acid; 9: Caffeic acid; 10: Vanillin; 11: *p*-Coumaric acid; 12: Sinapic acid; 13: Trans-Ferulic acid; 14: Ellagic acid; 15: Rutin; 16: Salicylic acid; 17: Quercetin; 18: Kaempfeol

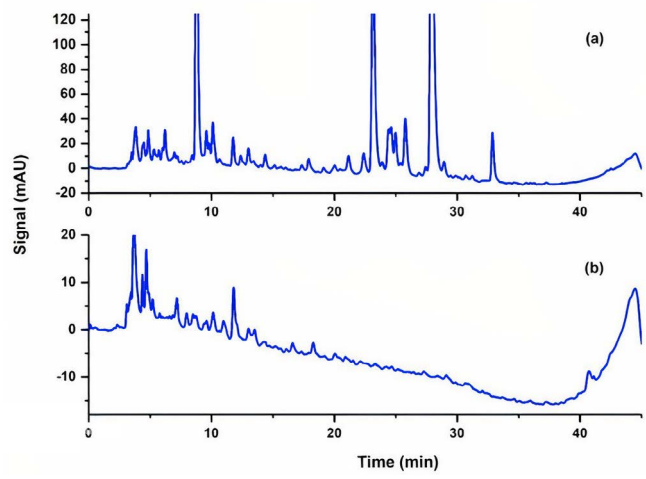

Fig. 2. Representative HPLC chromatograms of the phenolic compounds of the *G. cordifolium* leaf MeOH extract (a) and water extract (b)

(IC_{50}: 0.28±0.016 mg/mL), and the leaf methanol extracts (0.70±0.009 mg/mL). The ABTS radical scavenging activity of the extracts mainly demonstrated the following order: BHT (IC_{50}: 29.37±0.64 µg/mL) > the stem methanol extract (IC_{50}: 55.24±0.48 µg/mL) > the flower methanol extract (IC_{50}: 59.29±0.81 µg/mL).

The ferrous ion chelating activity of the extracts was evaluated using the ferrous-ferrozine method and the results were compared with the standard EDTA compound. The stem methanol extract demonstrated the strongest iron chelating activity (IC_{50}: 0.97±0.009 mg/

Table 2. The antioxidant activity of the *G. cordifolium* extracts*

Plant parts	Extracts	DPPH (IC_{50} mg/mL)	ABTS (IC_{50} µg/mL)	TPC (mg GAE/g)	TFC (mg QE/g)	Metal chelating activity (IC_{50} mg/mL)	β-Carotene / linoleic acid Emulsion method (IC_{50} mg/mL)
Flower	Methanol	0.25 ± 0.005^{g}	59.29 ± 0.81^{c}	137.52 ± 1.11	155.40 ± 5.94	3.62 ± 0.012^{c}	0.81 ± 0.005^{c}
	Water	12.9 ± 0.011^{b}	311.31 ± 1.36^{f}	45.22 ± 4.45	38.57 ± 2.94	1.23 ± 0.004^{g}	1.33 ± 0.015^{a}
Leaf	Methanol	0.70 ± 0.009^{f}	284.71 ± 1.23^{e}	53.07 ± 3.14	81.51 ± 1.36	4.03 ± 0.006^{b}	n.a.
	Water	22.4 ± 0.010^{a}	276.98 ± 1.26^{d}	24.53 ± 2.77	13.77 ± 2.27	2.23 ± 0.013^{d}	0.66 ± 0.070^{e}
Stem	Methanol	0.28 ± 0.016^{g}	55.24 ± 0.48^{b}	115.63 ± 2.43	144.63 ± 2.35	0.97 ± 0.009^{h}	0.55 ± 0.001^{f}
	Water	4.86 ± 0.018^{c}	467.47 ± 1.49^{i}	55.50 ± 5.27	36.57 ± 2.94	1.96 ± 0.016^{e}	1.27 ± 0.005^{b}
Root	Methanol	2.14 ± 0.005^{e}	421.05 ± 1.24^{g}	20.27 ± 2.07	4.07 ± 1.23	4.99 ± 0.056^{a}	0.76 ± 0.009^{d}
	Water	3.07 ± 0.075^{d}	443.54 ± 0.70^{h}	17.83 ± 0.59	11.09 ± 2.27	1.32 ± 0.015^{f}	0.56 ± 0.017^{f}
Quercetin		0.047 ±0.004^{g}					
BHT			29.37 ± 0.64^{a}				
EDTA						0.52 ± 0.006^{i}	
Trolox							0.85 ± 0.021^{c}

*The values expressed are Means±S.D. of three parallel measurements; n.a.: not active. The superscript letters in each column exhibit significant differences in the mean values at $p < 0.05$ according to the statistical analysis of Duncan's Multiple Comparison test.

Table 3. The enzyme inhibitory effect of the *G. cordifolium* extracts*

Plant parts	Extracts	AChE (mg Gala E/g)	BChE (mg Gala E/g)	Amylase (mg AcarE/g)	Glucosidase (mg AcarE/g)	Tyrosinase (mg KojE/g)
Flower	Methanol	0.17 ± 0.01^{f}	3.69 ± 0.02^{b}	4.09 ± 0.02^{c}	10.57 ± 0.02^{a}	1.64 ± 0.04^{bc}
	Water	0.25 ± 0.02^{e}	2.15 ± 0.03^{g}	7.52 ± 0.03^{a}	10.44 ± 0.03^{c}	1.60 ± 0.03^{cde}
Leaf	Methanol	0.32 ± 0.08^{e}	2.34 ± 0.11^{f}	1.09 ± 0.01^{e}	10.53 ± 0.04^{ab}	1.59 ± 0.01^{de}
	Water	1.02 ± 0.03^{b}	3.20 ± 0.05^{d}	3.43 ± 0.01^{d}	10.34 ± 0.09^{d}	1.57 ± 0.04^{e}
Stem	Methanol	0.40 ± 0.03^{d}	4.12 ± 0.03^{a}	5.04 ± 0.01^{b}	10.54 ± 0.01^{ab}	1.52 ± 0.01^{f}
	Water	0.60 ± 0.06^{c}	3.18 ± 0.02^{d}	4.49 ± 0.01^{c}	10.08 ± 0.06^{e}	1.65 ± 0.01^{b}
Root	Methanol	0.13 ± 0.03^{f}	3.34 ± 0.01^{c}	8.91 ± 0.05^{e}	10.33 ± 0.05^{d}	1.78 ± 0.02^{a}
	Water	1.61 ± 0.05^{a}	2.43 ± 0.05^{e}	4.63 ± 0.05^{c}	10.47 ± 0.02^{bc}	1.63 ± 0.01^{bcd}

*The values expressed are Means±S.D. (n=3); Gala E, galanthamine equivalents; AcarE, acarbose equivalents; KojE, kojic acid equivalents; AChE, acetylcholinesterase; BChE, Butyrylcholinesterase; $^{a\text{-}e}$ Means with different letters within each row are significantly different ($p < 0.05$)

Table 4. The percentage inhibition in browning, fructosamine content, carbonyl content, and aggregation of protein by the aqueous extracts of the different parts of *G. cordifolium*.

The extracts	Browning	Fructosamine content	Carbonyl content	Aggregation of protein
G. *cordifolium* (leaves)	54.4 ± 0.001^{5a}	9.73 ± 0.002^{a}	32.57 ± 0.01^{a}	53.72 ± 0.0005^{b}
G. cordifolium (flowers)	22.11± 0.003^{d}	1.26 ± 0.002^{d}	12.54 ± 0.006^{d}	43.09 ± 0.0010^{d}
G. cordifolium (stems)	46.43 ± 0.003^{c}	7.56 ± 0.012^{b}	30.35 ± 0.002^{b}	45.37 ± 0.0015^{c}
G. cordifolium (roots)	52.04 ± 0.0025^{b}	6.57 ± 0.0015^{c}	26.50 ± 0.002^{c}	81.68 ± 0.0025^{a}

The relative percentage was plotted using BSA + F as standard (100%). BSA: Bovine Serum Albumin, F: Fructose. The results are expressed as mean±SEM (n=3). Statistical significance $p < 0.05$ when compared to BSA + Fructose.

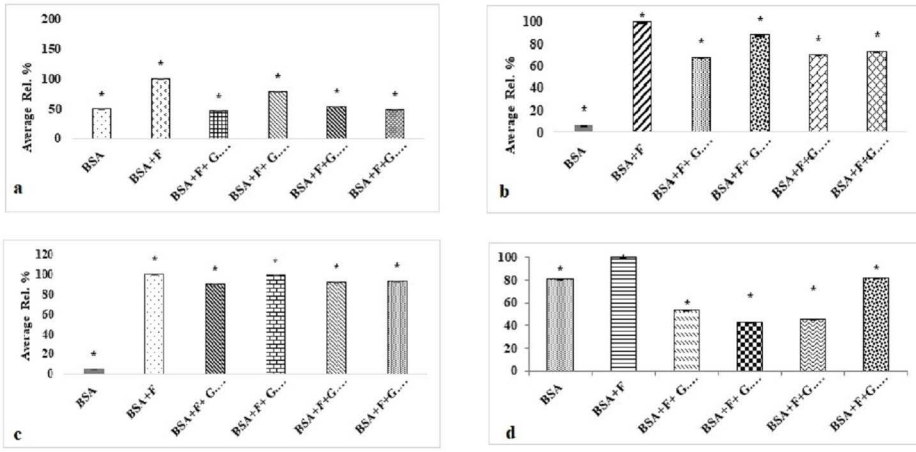

Fig. 3. Measurement of anti-glycation activity; (a) Browning; (b) Carbonyl content; (c): Fructosamine; (d): Protein aggregation.

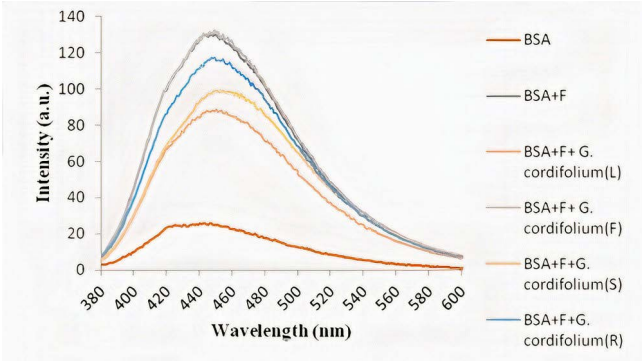

Fig. 4. Total AGEs formation.

mL) followed by the aqueous extracts from the flowers (IC_{50}: 1.23±0.004 mg/mL), and roots (IC_{50}: 1.32±0.015 mg/mL).

The β-carotene/linoleic acid emulsion method was used to measure the ability of an antioxidant to inhibit lipid peroxidation. In this method, the β-carotene/linoleic acid emulsion undergoes a rapid discoloration in the absence of antioxidants in the extract. In a previous study, amongst the tested fern extracts, *Dicranopteris linearis* exhibited the highest β-carotene bleaching activity of 61% at 0.1 mg/mL and increased to 99% at 0.7 mg/mL (Lai *et al.* 2011). In our study, the antioxidant activity also increased by increasing the concentration of the tested extracts. The stem methanol extract and root water extract exhibited the highest β-carotene bleaching antioxidant activity with IC_{50} values of 0.55±0.001 and 0.56±0.017 mg/mL. In another study, where *Coriandrum sativum* L. (coriander) and *Pimpinella anisum* L. belonging to the Apiaceae family were investigated for antioxidant activity by the β-carotene bleaching assay, anise and coriander showed 50% of β-carotene bleaching activity at 530±7 and 802±95 μg/mL of extract concentration (Martins *et al.* 2016).

Enzyme inhibition activity. The methanol and aqueous extracts obtained from different parts of *G. cordifolium* were evaluated for their enzyme inhibition activity such as acetylcholinesterase and butyrylcholinesterase related to neurodegenerative disorders, α-amylase and α-glucosidase related to diabetes mellitus, and tyrosinase related to skin melanogenesis and Parkinson's disease. In the present study, all of the tested extracts exhibited cholinesterase inhibitory activity. However, the aqueous extract of the roots (1.61mg Gala E/g) and the leaves (1.02 mg Gala E/g) showed the greatest acetylcholinesterase inhibitory effects. Moreover, the methanol extract of the stems exhibited the highest butyrylcholinesterase inhibitory effect (4.12 mg Gala E/g) (Table 3). All the extracts showed an almost similar effect against α-glucosidase and tyrosinase. The root methanol extract demonstrated the highest a-amylase inhibitory activity (8.91±0.05 mg AcarE/g), followed by the flower water extract (7.52±0.03 mg AcarE/g).

Anti-glycation activity

Effects of the *G. cordifolium* extracts on browning. It is evident from Table 4 and Fig. 3 that the aqueous extract of the *G. cordifolium* leaves caused a significant decrease of 54.4% in the browning of BSA by fructose followed by the root extract (52.04%), stem extract (46.43%) and the flower extract (22.11%).

Effects of the *G. cordifolium* extracts on fructosamine production. A comparison of the amount of fructosamine between the glycated sample in the presence and in the absence of the *G. cordifolium* extracts showed that all the extracts were efficient in preventing the formation of fructosamine with respect to the control (BSA + Fructose) (Fig. 3c). The *G. cordifolium* leaf extract caused a 9.73% decrease, while the flower extract showed only a 1.26% decrease in the amount of fructosamine content formation as compared to glycated protein (Table 4).

Effects of the *G. cordifolium* extracts on the carbonyl content. The amount of carbonyl content was measured in the glycated sample in both the presence and absence of the aqueous extracts of different parts of *G. cordifolium* by the DNPH method. It was observed that the carbonyl content was reduced by 32.57% in the presence of the leaf extract of *G. cordifolium*, and by 30.35%, 26.50%, and 12.54% in the case of the stem, root, and flower extracts respectively, as compared to glycated BSA (Table 2, Fig. 3).

Effects of the *G. cordifolium* extracts on protein aggregation. Protein aggregation is the late stage in the non-enzymatic glycation process in which the binding of the carbonyl group to the amino group of proteins takes place leading to the formation of clusters. Here, it leads to a significant reduction of the amyloid cross-β structure in the presence of the extracts in comparison to fructose glycated protein as shown in Table 4. The highest inhibition was observed by the aqueous extract of the *G. cordifolium* roots (Fig. 3d). The amount of protein aggregation flows in the order of root extract > leaf extract > stem extract > flower extract.

Effects of the *G. cordifolium* extracts on total advanced glycation and product formation. It can be seen from Fig. 4 that all the plant extracts, with the exception of the aqueous extract of the *G. cordifolium* flowers drastically reduced the number of AGEs as compared to fructated protein (BSA + Fructose). The results established that the addition of the aqueous extracts of the leaf into the solution greatly reduced the formation of fluorescent AGE (Fig. 4).

DISCUSSION

The total phenolic contents in the extracts were calculated from the regression equation of the gallic acid calibration curve and expressed in terms of mg gallic acid equivalents (GAE) per g dry weight of the extract (DW). The amount of total phenolic compounds ranged from 17.83±0.59 to 137.52±1.11 mg GAE/g DW. The total flavonoid contents of the extracts were evaluated by the aluminum chloride colorimetric method and the amounts were expressed as quercetin (QE) equivalents and ranged from 4.07±1.23 to 155.40±5.94 mg QE/g DE. Among the extracts, the flower methanol extract showed the highest phenolic and flavonoid compounds. Moreover, these results are also compatible with the obtained HPLC results.

According to the HPLC analysis, the aqueous extract of the roots was found to be rich in phenolic acids such as caffeic acid, and 1,2-dihydroxy benzene, which may be responsible for the acetylcholinesterase inhibitory activity (Anwar *et al.* 2012). Caffeic acid has been reported for anti-AChE and anti-BChE activity. Polyphenolic compounds have been shown to demonstrate cholinesterase inhibitory activity depending on the number and position of their OH groups, which create hydrogen bonds with certain amino acids at the enzyme's active sites (Oboh *et al.* 2013). As for the methanol extract of the stems, it demonstrated strong DPPH and ABTS radical scavenging, iron chelating, and β-carotene decolorization activity, and had high amounts of total phenol and flavonoid contents according to the spectrophotometric and HPLC results. In this sense, the butyrylcholinesterase inhibitory activity of the extract may be attributed to its rich phenolic content and significant antioxidant activity. Several studies of phytochemicals also described the anticholinesterase effects of many flavonoids and their derivatives (Uriarte-Pueyo & Calvo 2011; Anand & Singh 2013).

Antioxidants present in nutrition are important in maintaining the oxidant-antioxidant balance in human tissues. Natural antioxidants from medicinal plants can protect cells against oxidative and electrophilic stress caused by reactive oxygen species, which may lead to the damage of DNA, protein and cell membranes, related to many diseases (Ebisch *et al.* 2007). Plant extracts exhibit antioxidant activities via different kind of mechanisms because of the different functional groups of compounds they contain. In a previous study, the essential oil obtained from the aerial part, fruit and roots of *G. cordifolium* showed DPPH radical scavenging activity with IC_{50} values of 1.14±0.086, 1.02±0.07 and 1.18±0.052 mg/ml, respectively and ABTS radical scavenging activity with IC_{50} values of 0.94±0.075, 1.01±0.069 and 1.09±0.075 mg/ml, respectively (Karadag *et al.* 2019).

Metal ions are involved in the lipid peroxidation chain reaction which causes the deterioration of foods. On the other hand, they are closely related with the occurrence of some diseases such as cancer, and arthritis (Sarikürkcü *et al.* 2010). Therefore, antioxidant activity can be evaluated by measuring the chelating capacity of plant extracts on metal ions such as iron and copper. In this study, the stem methanol extract showed the highest iron chelating activity. The same extract also showed the highest anti-butyrylcholinesterase activity. It can thus be seen that there is a relation between metal chelation and cholinesterase activity. This view is supported by previous studies (Cherny *et al.* 2001). Therefore, iron chelation has been proposed as a potential therapy in the treatment of neurodegenerative illnesses (Nunez & Chana-Cuevas 2018).

Anticholinesterase is one of the most attractive targets for drug intervention to treat neurodegenerative diseases due to its essential catalytic role in the hydrolysis of acetylcholine into choline and acetyl. According to the cholinergic deficit hypothesis, the reduced level of acetylcholine caused by cholinesterase hyperactivity is closely related to the degeneration of cholinergic function in the brain which subsequently causes Alzheimer's diseases. There is great interest in finding a new potential natural cholinesterase inhibitor without side effects since

the currently used inhibitors, such as tacrine, donepezil, and rivastigmine, can cause some side effects, including hepatotoxicity, diarrhoea, and gastrointestinal disturbances (Sandra Gonçalves 2017). In a study on *Peucedanum ostruthium* from the Apiaceae family which is close to our species, four coumarins were isolated and ostruthol was determined as the strongest pure compound against AChE by a TLC bioautographic assay with a 0.001 µg minimum inhibitory quantity (Urbain *et al.* 2005). In another study, where fifteen khellactone coumarin compounds isolated from *Peucedanum japonicum* were analysed for their abilities to inhibit AChE, BChE, MAO-A, and MAO-B, 3'-angeloyl-4'-(2-methylbutyryl)khellactone and senecioyl-4'-angeloyl-khellactone were the most efficient in inhibiting AChE (IC_{50} = 9.28 µM) and BChE (IC_{50} = 7.22 µM), respectively (Heo *et al.* 2020).

DM is a serious medical concern due to its complications including cardiovascular disease, hypertension, kidney failure, blindness, and neurological disorders (López-Candales 2001). One of the strategic treatments of DM is to decrease the postprandial glucose level, which can be possible with the carbohydrate hydrolyzing enzymes α-amylase, and α-glucosidase. Inhibitors of these enzymes can delay the rate of glucose absorption and thus serve as an important clinical therapeutic approach for controlling hyperglycemia. However, the α-amylase, and α-glucosidase inhibitor drug acarbose, which is approved by the FDA, can cause severe side effects. Therefore, it is necessary to find out potential alternatives of natural origin. According to the results of our study, the methanol extract of the roots (8.91 mg Acar E/g) showed the greatest α-amylase inhibitory effect, while the methanol extract of the flowers (10.57 mg Acar E/g) showed the greatest α-glucosidase inhibitory effect. Among the secondary metabolites, flavonoids have also shown the capacity to inhibit both α-amylase and α-glucosidase (Tadera *et al.* 2006; Hua *et al.* 2018). As for our phytochemical results, the methanol extract of the roots and flowers mainly contained phenolic compounds. Inhibition of α-glucosidase and pancreatic amylase by different classes of phenolic compounds is also described in the literature (Shobana *et al.* 2009; Oboh *et al.* 2016; Kalita *et al.* 2018).

Searching for tyrosinase inhibitors is important for developing anti-browning substances for foods as well as skin whitening agents. To date, several tyrosinase inhibitors have been reported for their potential activity from natural sources, especially from plants (Souza *et al.* 2012). In this study, although all the tested extracts exhibited tyrosinase inhibitory activity, there was not much difference between them. Phenolic compounds have been shown to form hydrogen bonds with active sites of tyrosinase enzymes to promote steric hindrance, and conformational change, and directly suppress enzyme activity. Tyrosinase inhibitory activity is closely associated with a hydroxyl group of phenolic compounds (Sun *et al.* 2017). AGEs are being investigated as the possible cause of a variety of disorders, and current research has concentrated on compounds which limit AGE synthesis and AGE binding in tissues (AGEs-protein cross-link). ROS accelerates the production of AGE caused by hyperglycemia and nonenzymatic glycosylation of proteins (Yang *et al.* 2019). Furthermore, binding and interaction with the AGE receptor (RAGE) causes cell damage by activating downstream signaling which regulates not just ROS-related pathways but also cell differentiation and death (Sparvero *et al.* 2009). As a result, it was assumed that developing materials which limit the formation of AGEs and break crosslinks, as well as high-efficiency antioxidants, would be critical for the treatment and prevention of AGE-related disorders such as diabetic complications.

CONCLUSION

In conclusion, our results show that the methanol extracts from the flowers, leaves, roots, and stems of *G. cordifolium* had different enzyme inhibitory activity. These inhibitory effects can be comparable with standard inhibitors. In addition, the plant extracts also demonstrate the highest radical scavenging activity, which may be attributed to their high amounts of total polyphenols and flavonoids. These results suggest that methanol and aqueous extracts of *G. cordifolium* may be useful as a source of bioactive compounds for developing new alternative drugs to the current pharmaceutical formulations against oxidative stress, as well as for natural enzyme inhibitors. The results presented also show that all the aqueous extracts of *G. cordifolium* exhibited significant antiglycation potential. The highest inhibition was caused by the aqueous extract of the *G. cordifolium* leaves, and the lowest inhibition was shown by the aqueous extract of the *G. cordifolium* flowers, while the aqueous extracts of the *G. cordifolium* stems and roots showed moderate inhibition. According to our outcomes, it may be deduced that these aqueous plant extracts can inhibit the process of glycation by various approaches such as (i) inhibiting the early glycation product formation (fructosamine), (ii) reducing the generation of reactive carbonyl/dicarbonyl groups and (iii) inhibiting AGE formation. The results of our study also suggest the protective effect of polyphenolic compounds found in the extracts against glycation-induced cellular damage and inhibition of AGEs.

Acknowledgements – The author would like to thank the Selcuk University Scientific Research Foundation (SUBAP-18401141) and the University of Mumbai for their financial support.

REFERENCES

Abirami A, Nagarani G & Siddhuraju P. 2014. In vitro antioxidant, anti-diabetic, cholinesterase and tyrosinase inhibitory potential of fresh juice from *Citrus hystrix* and *C. maxima* fruits. *Food Science and Human Wellness* **3**: 16–25.

Adisakwattana S, Thilavech T & Chusak C. 2014. Mesona Chinensis Benth extract prevents AGE formation and protein oxidation against fructose-induced protein glycation in vitro. *BMC Complementary and Alternative Medicine* **14**: 1–9.

Ahmad S, Shahab U, Habib S, Salman Khan M, Alam K & Ali A. 2014. Glycoxidative damage to human DNA: Neo-antigenic epitopes on DNA molecule could be a possible reason for autoimmune response in type 1 diabetes. *Glycobiology* **24**: 281–291.

Aldini G, Vistoli G, Stefek M, Chondrogianni N, Sereikaite J, Sadowska-Bartosz I & Bartosz G. 2013. Molecular strategies to prevent, inhibit, and degrade advanced glycoxidation and advanced lipoxidation end products. *Free Radical Research* **47**: 93–137.

Ali A, More TA, Hoonjan AK & Sivakami S. 2017. Antiglycating potential of acesulfame potassium: an artificial sweetener. *Applied Physiology, Nutrition, and Metabolism* **42**: 1054–1063.

Ali A, Shahu R, Balyan P, Kumari S, Ghodmare R, Jobby R & Jha P. 2022. Antioxidation and antiglycation properties of a natural sweetener: *Stevia rebaudiana*. *Sugar Tech* **24**: 563–575.

Anand P & Singh B. 2013. Flavonoids as lead compounds modulating the enzyme targets in Alzheimer's disease. *Medicinal Chemistry Research* **22**: 3061–3075.

Anwar J, Spanevello RM, Thomé G, Stefanello N, Schmatz R, Gutierres J, Vieira J, Baldissarelli J, Carvalho FB, da Rosa MM, Rubin MA, Fiorenza A, Morsch VM & Schetinger MR. 2012. Effects of caffeic acid on behavioral parameters and on the activity of acetylcholinesterase in different tissues from adult rats. *Pharmacology Biochemistry and Behavior* **103**: 386–394.

Banan P & Ali A. 2016. Preventive effect of phenolic acids on in vitro glycation. *Annals of Phytomedicine* **5**: 97–102.

Başer KHC, Özek T, Demirci B & Duman H. 2000. Composition of the essential oil of *Glaucosciadium cordifolium* (Boiss.) Burtt et Davis from Turkey. *Flavour and Fragrance Journal* **15**(1): 45–46.

Bulut G, Tuzlaci E, Doğan A & Şenkardeş İ. 2014. An ethnopharmacological review on the Turkish Apiaceae species. *Istanbul Journal of Pharmacy* **44**: 163–179.

Chai T, Mohan M, Ong H & Wong F. 2014. Antioxidant, iron-chelating and anti-glucosidase activities of *Typha domingensis* Pers (Typhaceae). *Tropical Journal of Pharmaceutical Research* **13**: 67–72.

Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jones WD, McLean CA, Barnham KJ, Volitakis I, Fraser FW, Kim YS & Huang X. 2001. Treatment with a copper-zinc chelator markedly and rapidly inhibits β-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* **30**: 665–676.

Clarke G, Ting K, Wiar C & Fry J. 2013. High correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. *Antioxidants* **2**: 1–10.

di Petrillo A, González-Paramás AM, Era B, Medda R, Pintus F, Santos-Buelga C & Fais A. 2016. Tyrosinase inhibition and antioxidant properties of *Asphodelus microcarpus* extracts. *BMC Complementary and Alternative Medicine* **16**: 1–9.

Ebisch IMW, Thomas CMG, Peters WHM & Braat DDM. 2007. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. *Human Reproduction Update* **13**: 163–174.

Eruygur N & Ayaz F. 2021. Investigation of acetylcholinesterase, butyrylcholinesterase, α-glucosidase, α-amylase, and tyrosinase inhibition and antioxidant activity of methanol and water extracts from aerial parts of *Phlomis lycia* D. DON. *International Journal of Phytocosmetics and Natural Ingredients* **8**(1): 3.

Eruygur N, Kirci D, Ayaz F, Dogu S & Bagci Y. 2022. Biological activities of three *Phlomis* species. *Journal of Research in Pharmacy* **26**: 255–262.

Gonçalves S, Moreira E, Grosso C, Andrade PB, Valentão P & Romano A. 2017. Phenolic profile, antioxidant activity and enzyme inhibitory activities of extracts from aromatic plants used in Mediterranean diet. *Journal of Food Science and Technology* **54**(1): 219–227.

Hedge IC & Lamond JM. 1972. *Glaucosciadium* Burtt & Davis. In: Davis PH (ed.), *Flora of Turkey and the East Aegean Islands*, vol **4**, pp. 513–514, Edinburgh University Press, Edinburgh.

Heo JH, Eom BH, Ryu HW, Kang MG, Park JE, Kim DY, Kim JH, Park D, Oh SR & Kim H. 2020. Acetylcholinesterase and butyrylcholinesterase inhibitory activities of khellactone coumarin derivatives isolated from *Peucedanum japonicum* Thurnberg. *Scientific Reports* **10**: 1–11.

Hua F, Zhou P, Wu HY, Chu GX, Xie ZW & Bao GH. 2018. Inhibition of flavonoid glycosides from Lu'an GuaPian tea on α-glucosidase and α-amylase: molecular docking and interaction mechanism. *Journal of Functional Foods* **9**: 4173–4183.

Jeong SH, Ryu YB, Curtis-Long MJ, Ryu HW, Baek YS, Kang JE, Lee WS & Park KH. 2009. Tyrosinase inhibitory polyphenols from roots of *Morus lhou*. *Journal of Agricultural and Food Chemistry* **57**: 1195–1203.

Kalita D, Holm DG, Labarbera DV, Petrash JM & Jayanty SS. 2018. Inhibition of α-glucosidase, α-amylase, and aldose reductase by potato polyphenolic compounds. *Plos One* **13**: e0191025.

Karadağ AE, Demirci B, Çeçen Ö & Tosun F. 2019. Chemical characterization of *Glaucosciadium cordifolium* (Boiss.) B.L.Burtt & P.H.Davis essential oils and their antimicrobial, and antioxidant activities. *Istanbul Journal of Pharmacy* **49**: 77–80.

Karadoğan T, Şanli A, Tosun B & Özçelik H. 2015. Essential oil content and composition of *Glaucosciadium cordifolium* (Boiss) Burtt & Davis from Lakes region. *Research Journal of Biological Sciences* **8**: 35–39.

Kubo I, Chen QX, Nihei KI, Calderón JS & Céspedes CL. 2003. Tyrosinase inhibition kinetics of anisic acid. *Zeitschrift für Naturforschung* **58**: 713–718.

Kumar D, Bhatkalkar SG, Sachar S & Ali A. 2021. Studies on the antiglycating potential of zinc oxide nanoparticles and its interaction with BSA. *Journal of Biomolecular Structure and Dynamics* **39**: 6918–6925.

Lai H & Lim Y. 2011. Evaluation of antioxidant activities of methanolic extracts of selected ferns in Malaysia. *International Journal of Environmental Science and Development* **2**: 442–447.

López-Candales A. 2001. Metabolic syndrome X: A comprehensive review of the pathophysiology and recommended therapy. *Journal of Medicine* **32**: 283–300.

Martins N, Barros L, Dueñas M, Santos-Buelga C & Ferreira IC. 2015. Characterization of phenolic compounds and antioxidant properties of *Glycyrrhiza glabra* L. rhizomes and roots. *RSC Advances* **5**: 26991–26997.

Martins N, Barros L, Santos-buelga C & Ferreira IC. 2016. Antioxidant potential of two Apiaceae plant extracts: A comparative study focused on the phenolic composition. *Industrial Crops and Products* **79**: 188–194.

Meeprom A, Sompong W, Chan CB & Adisakwattana S. 2013. Isoferulic acid, a new anti-glycation agent, inhibits fructose- and glucose- mediated protein glycation in vitro. *Molecules* **18**: 6439–6454.

Nuñez MT & Chana-Cuevas P. 2018. New perspectives in iron chelation therapy for the treatment of neurodegenerative diseases. *Pharmaceuticals* **11**: 109.

Oboh G, Agunloye OM, Akinyemi AJ, Ademiluyi AO & Adefegha SA. 2013. Comparative study on the inhibitory effect of caffeic and chlorogenic acids on key enzymes linked to Alzheimer's disease and some pro-oxidant induced oxidative stress in rats' brain in vitro. *Neurochemical Research* **38**: 413–419.

Oboh G, Ogunsuyi OB, Ogunbadejo MD & Adefegha SA. 2016. Influence of gallic acid on α -amylase and α -glucosidase inhibitory properties of acarbose. *Journal of Food and Drug Analysis* **24**: 1–8.

Özek G. 2018. Chemical diversity and biological potential of *Tanacetum praeteritum* subsp. *praeteritum* essential oils. *Journal of the Turkish Chemical Society Section* A **5**: 493–510.

Pires C, Martins N, Carvalho AM, Barros L & Ferreira IC. 2016. Phytopharmacological preparations as predictors of plant bioactivity: A particular approach to *Echinacea purpurea* (L.) Moench antioxidant properties. *Nutrition* **32**: 834–839.

Qurrat-ul-Ain, Ashiq U, Jamal RA, Saleem M & Mahroof-Tahir M. 2017. Alpha-glucosidase and carbonic anhydrase inhibition studies of Pd (II)-hydrazide complexes. *Arabian Journal of Chemistry* **10**(4): 488–499.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M & Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* **26**: 1231–1237.

Rhee IK, van de Meent M, Ingkaninan K & Verpoorte R. 2001. Screening for acetylcholinesterase inhibitors from Amaryllidaceae using silica gel thin-layer chromatography in combination with bioactivity staining. *Journal of Chromatography A* **915**: 217–223.

Roy A, Sen S & Chakraborti AS. 2004. In vitro nonenzymatic glycation enhances the role of myoglobin as a source of oxidative stress. *Free Radical Research* **38**: 139–146.

Russo D, Valentão P, Andrade PB, Fernandez EC & Milella L. 2015. Evaluation of antioxidant, antidiabetic and anticholinesterase activities of *Smallanthus sonchifolius* landraces and correlation with their phytochemical profiles. *International Journal of Molecular Sciences* **16**: 17696–17718.

Salehi P, Asghari B, Esmaeili MA, Dehghan H & Ghazi I. 2013. α-glucosidase and α-amylase inhibitory effect and antioxidant activity of ten plant extracts traditionally used in Iran for diabetes. *Journal of Medicinal Plants Research* **7**: 257–266.

Sarikurkcu C, Tepe B, Semiz DK & Solak MH. 2010. Evaluation of metal concentration and antioxidant activity of three edible mushrooms from Muğla, Turkey. *Food and Chemical Toxicology* **48**: 1230–1233.

Shobana S, Sreerama YN & Malleshi NG. 2009. Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: Mode of inhibition of α-glucosidase and pancreatic amylase. *Food Chemistry* **115**: 1268–1273.

Singh VP, Bali A, Singh N & Jaggi AS. 2014. Advanced glycation end products and diabetic complications. *The Korean Journal of Physiology & Pharmacology* **18**: 1–14.

Souza PM, Elias ST, Simeoni LA, de Paula JE, Gomes SM, Guerra ENS & Magalhaes PO. 2012. Plants from Brazilian Cerrado with potent tyrosinase inhibitory activity. *PloS One* **7**: e48589.

Sparvero LJ, Asafu-adjei D, Kang R, Tang D, Amin N, Im J, Rutledge R, Lin B, Amoscato AA, Zeh HJ & Lotze MT. 2009. RAGE (Receptor for Advanced Glycation End products), RAGE ligands, and their role in cancer and inflammation. *Journal of Translational Medicine* **7**(1): 1–21.

Sun L, Guo Y, Zhang Y & Zhuang Y. 2017. Antioxidant and anti-tyrosinase activities of phenolic extracts from rape bee pollen and inhibitory melanogenesis by cAMP / MITF / TYR Pathway in B16 Mouse Melanoma. *Frontiers in Pharmacology* **8**: 1–9.

Tadera K, Minami Y, Takamatsu K & Matsuoka T. 2006. Inhibition of α-glucosidase and α-amylase by flavonoids. *Journal of Nutritional Science and Vitaminology* **52**: 149–53.

Telagari M & Hullatti K. 2015. In-vitro α-amylase and α-glucosidase inhibitory activity of *Adiantum caudatum* Linn. and *Celosia argentea* Linn. extracts and fractions. *Indian Journal of Pharmacology* **47**: 425–429.

Urbain A, Marston A & Hostettmann K. 2005. Coumarins from *Peucedanum ostruthium* as inhibitors of acetylcholinesterase. *Pharmaceutical Biology* **43**: 647–650.

Uriarte-Pueyo I & Calvo MI. 2011. Flavonoids as acetylcholinesterase inhibitors. *Current Medicinal Chemistry* **18**: 5289–5302.

Yang H, Dong Y, Du H, Shi H, Peng Y & Li X. 2011. Antioxidant compounds from propolis collected in Anhui, China. *Molecules* **16**: 3444–3455.

Yang P, Feng J, Peng Q, Liu X & Fan Z. 2019. Advanced glycation end products: potential mechanism and therapeutic target in cardiovascular complications under diabetes. *Oxidative Medicine and Cellular Longevity* 2019: 9570616.

Yikna BB & Yehualashet AS. 2021. Medicinal plant extracts evaluated in vitro and in vivo for antidiabetic activities in Ethiopia: bases for future clinical trials and related investigations. *Evidence-Based Complementary and Alternative Medicine* **2021**: 9108499.

REZIME

Botanica SERBICA

Ispitivanje fenolnih jedinjenja, *in vitro* antioksidativnih i enzimskih inhibicionih aktivnosti metanolnih i vodenih ekstrakata različitih delova *Glaucosciadium cordifolium*

Nuraniye Eruygur, Fatma Ayaz, Yavuz Bağci, Hamide Filiz Ayyildiz, Esra Maltaş Çağil, Prairna Malik i Ahmad Ali

Ova studija je osmišljena da proceni biološke potencijale i fenolni sastav različitih delova *Glaucosciadium cordifolium*, koji je manje istražen i poznat kao divlji endemit Turske. Antioksidativna aktivnost biljke određena je korišćenjem 2,2-difenil-1-pikrilhidrazila (DPPH), 2,2'-azinobis-(3-etilbenztiazolin-6-sulfonske kiseline) (ABTS), helatnog kapaciteta gvožđa i b-karoten / test emulzije linolne kiseline. Ukupni sadržaj fenola i flavonoida u biljci određen je metodom Folin-Ciocalteu i aluminijum hloridom, respektivno. Ispitivanje aktivnosti inhibicije enzima biljke sprovedeno je za acetilholinesterazu, butirilholinesterazu, a-glukozidazu, a-amilazu i tirozinazu. Antiglikaciona aktivnost vodenog ekstrakta biljke je procenjena korišćenjem utvrđenih metoda kao što su posmeđivanje, nitroplavo-tetrazolijum (NBT) esej, 2,4-dinitrofenil hidrazin (DNPH) metoda, Kongo crveni i goveđi serum albumin (BSA)-fluorescentni test. HPLC profilisanje fenola je pokazalo da je 18 standardnih fenolnih jedinjenja pronađeno u različitim količinama u različitim ekstraktima delova biljke. Prema našim rezultatima bioaktivnosti, metanolni ekstrakt dobijen iz cvetnih delova biljke sadržao je veću količinu fenolnih jedinjenja i flavonoida, što je takođe pokazalo najveću aktivnost uklanjanja DPPH radikala. Inače, metanolni ekstrakti dobijeni iz listova i korena utvrđeni su kao najaktivniji ekstrakti protiv enzima acetilholinesteraze, kao i umereno aktivni protiv enzima tirozinaze. Kapacitet antiglikacije ekstrakta prati ovaj redosled: listovi *G. cordifolium* > stablo > koren > cvet. Kao rezultat toga, naša studija je pokazala da ekstrakti *G. cordifolium* imaju snažan antioksidativni potencijal, dobra inhibitorna dejstva enzima i antiglikacioni potencijal. Dalje studije o *G. cordifolium* sa *in vivo* biotestovima zahtevaju istraživanja kako bi se utvrdio značaj biljke u farmaceutskim tehnikama.

Ključne reči: *Glaucosciadium cordifolium*, HPLC analize, antioksidativna aktivnost, inhibicija enzima, antiglikaciona aktivnost